

Investigations of pulmonary epithelial cell damage due to air-liquid interfacial stresses in a microgravity environment

Donald P. Gaver III

Department of Biomedical Engineering, Tulane University, New Orleans, LA, USA

A.M. Bilek, S. Kay and K.C Dee

Department of Biomedical Engineering, Tulane University, New Orleans, LA, USA

Pulmonary airway closure is a potentially dangerous event that can occur in microgravity environments and may result in limited gas exchange for flight crew during long-term space flight. Repetitive airway collapse and reopening subjects the pulmonary epithelium to large, dynamic, and potentially injurious mechanical stresses. During ventilation at low lung volumes and pressures, airway instability leads to repetitive collapse and reopening. During reopening, air must progress through a collapsed airway, generating stresses on the airway walls, potentially damaging airway tissues. The normal lung can tolerate repetitive collapse and reopening. However, combined with insufficient or dysfunctional pulmonary surfactant, repetitive airway collapse and reopening produces severe lung injury. Particularly at risk is the pulmonary epithelium. As an important regulator of lung function and physiology, the degree of pulmonary epithelial damage influences the course and outcome of lung injury. In this paper we present experimental and computational studies to explore the hypothesis that the mechanical stresses associated with airway reopening inflict injury to the pulmonary epithelium.

Experimental Investigations

Experiments were performed in a parallel plate chamber lined with pulmonary epithelial cells, which was constructed as an idealized model of a collapsed segment of an airway where the walls are held in opposition by a viscous fluid. These experiments were conducted to determine whether air-liquid interfacial stresses can cause damage to epithelial cells, and to provide response behavior that can be correlated to the mechanical stimuli determined from computational investigations (below).

In a first set of experiments, a fetal rat pulmonary epithelial cell line (CCL-149, ATCC) was cultured to confluence on a small (1 cm^2), square region of the upper plate. The narrow channel was filled a model airway lining fluid. Phosphate buffered saline including 0.1 mg/mL CaCl_2 and MgSO_4 (PBS) was used to model a surfactant-deficient airway lining fluid. A surfactant-containing airway lining fluid was approximated using Infasurf (ONY, Inc.) diluted to 1 mg/mL phospholipid concentration in PBS. Airway “reopening” was generated by the steady progression of a semi-infinite bubble of air down the length of the channel using a constant rate infusion pump (7 or 70 mL/min). A digital camera mounted above the channel collected sequential overhead images of the progressing bubble, which were used to calculate bubble velocity. Once removed from the apparatus, the slide was incubated with $1.2 \text{ }\mu\text{M}$ Ethidium homodimer-1 (Eth-1) and $1.2 \text{ }\mu\text{M}$ calcein AM (Molecular Probes). For each slide, the number of injured cells was recorded as the average number of Eth-1 stained nuclei counted in fluorescence microscopic images.

In a second experimental study, we attempted to discriminate the stress magnitude from the stimulus duration. To do so, the stress magnitude is modified by varying the viscosity of the

occlusion fluid while fixing the reopening velocity across experiments. This approach causes the stimulus duration to be inversely related to the magnitude of the pressure gradient. We also explore the mechanism for acute damage and demonstrate that repeated reopening and closure is shown to damage the epithelial cell layer even under conditions that would not lead to extensive damage from a single reopening event.

Fluid Dynamic Simulations

The bubble and parallel-plate flow chamber was modeled as a semi-infinite bubble progressing within a Hele-Shaw cell. In this model the walls were separated by a distance $2H$, with the semi-infinite bubble progressing in the x -direction with tip velocity U . The surface tension, γ , was constant. The capillary number, $Ca = \mu U / \gamma$, representing the relative importance of viscous to surface tension effects on the bubble determines the dynamic response of the system. Stokes equations, $\nabla \mathbf{P} = \mu \nabla^2 \mathbf{u}$ and $\nabla \cdot \mathbf{u} = 0$, were solved using the boundary element method. The interfacial stress condition applied at the air-liquid interface was $|\boldsymbol{\sigma} \cdot \hat{\mathbf{n}}| = \gamma \kappa \hat{\mathbf{n}}$, where $\boldsymbol{\sigma} = -\mathbf{P}\mathbf{I} + \mu(\nabla \mathbf{u} + \nabla \mathbf{u}^T)$ was the stress tensor, $\hat{\mathbf{n}}$ was the unit normal, and κ was the interfacial curvature. For a given Ca , the system was simulated until a steady-state meniscus had developed and the stress-field and bubble geometry were determined.

Three potentially injurious components of the stress cycle associated with bubble progression – the shear stress, the shear stress gradient, and the pressure gradient – were analyzed. Regression relationships describing the behavior of these components as a function of Ca were determined for very small Ca ($5 \times 10^{-4} \leq Ca \leq 2 \times 10^{-3}$). Additionally, the thickness of the thin film deposited by bubble progression was estimated. Dimensionless values for the experimental flow conditions were extrapolated from the regression equations and redimensionalized.

Results and Discussion

For each condition the average number of injured cells per square centimeter was measured. For the saline-occluded channels, bubble progression at both velocities produced significantly increased numbers of injured cells when compared to the control. The slow velocity resulted in a 66-fold increase in the number of injured cells and the fast velocity produced a 20-fold increase. The addition of Infasurf to the occlusion fluid reduced the number of injured cells to a level similar to the control. These results support the hypotheses that mechanical stresses associated with airway reopening injure pulmonary epithelial cells and that pulmonary surfactant in the normal lung protects the epithelium from injury due to airway reopening.

The stress component that best agrees with the experimentally observed trauma is the maximal pressure gradient. Pressure gradients create a force imbalance on the cell membrane over the length of the cell. In addition, cell damage remains directly correlated with the pressure gradient, not the duration of stress exposure. For a low profile predominately flat cell (or region of a cell), the non-uniformly distributed load can depress the cell and stretch the membrane. For high profile cells or regions of a cell, such as the protrusion cause by the nucleus, where the normal forces of the cell surface are nearly opposite, the pressure gradient will pinch that region. The pinching can tear the membrane at the base of the protrusion or force fluid upward rupturing the top surface of the cell. The present study thus provides additional evidence that the magnitude of the pressure gradient induces cellular damage in this model of airway reopening.

Investigations of Pulmonary Epithelial Cell Damage Due to Air-Liquid Interfacial Stresses in a Microgravity Environment

Donald P. Gaver
Anastacia M. Bilek
Sarina Kay
Anne-Marie Jacob
Kay C Dee

Department of Biomedical Engineering
Tulane University
New Orleans, LA



Critical Path RoadMap

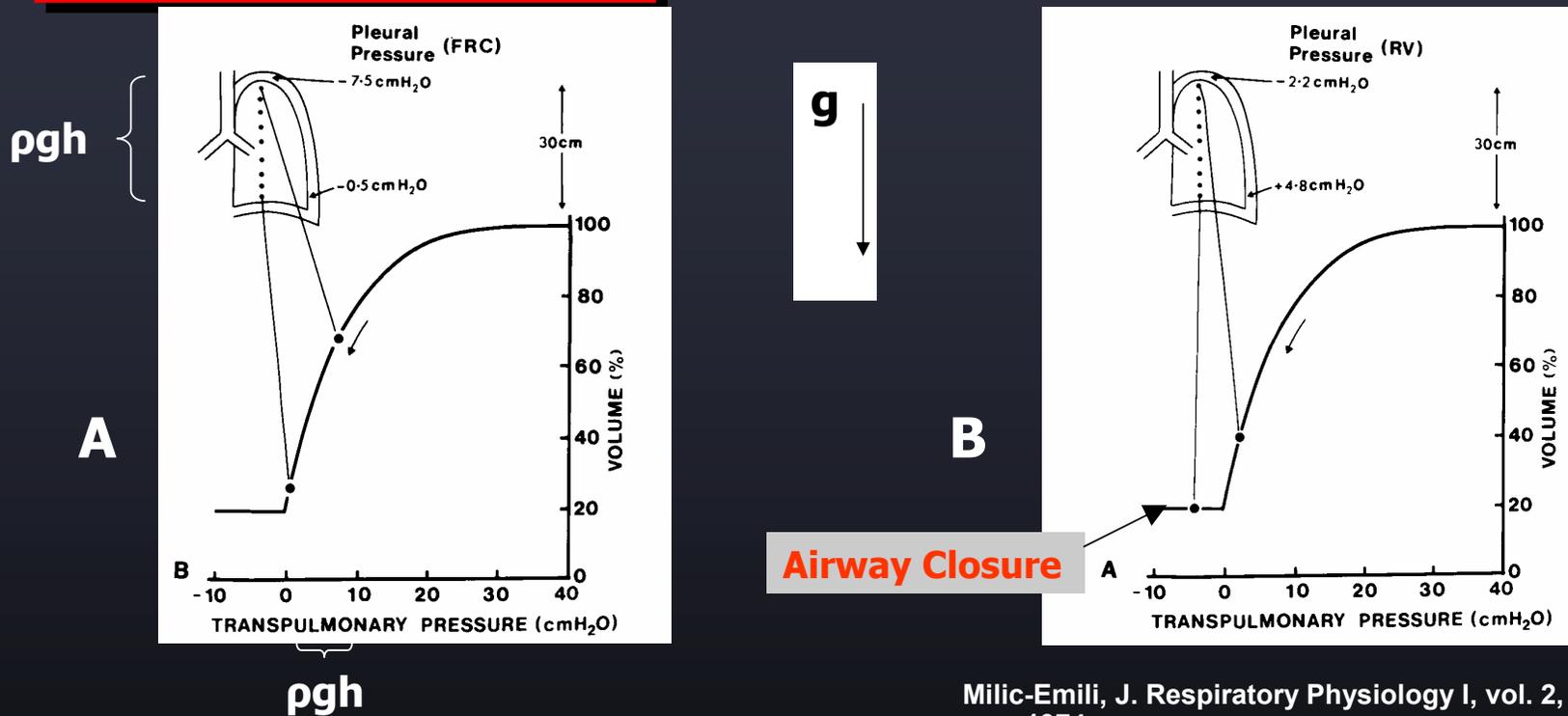
Cardiovascular Alterations

- Impaired Response due to Modified Orthostatic Mechanical Stress
- Diminished Cardiac Function
- Impaired Response to Exercise Stress

Pulmonary Alterations

- Airway Closure Becomes more Homogeneous
- Potentially Impaired Pulmonary Function
- Impaired Response to Exercise Stress

Gravity Effects on Ventilation Distribution



Milic-Emili, J. Respiratory Physiology I, vol. 2, 1974

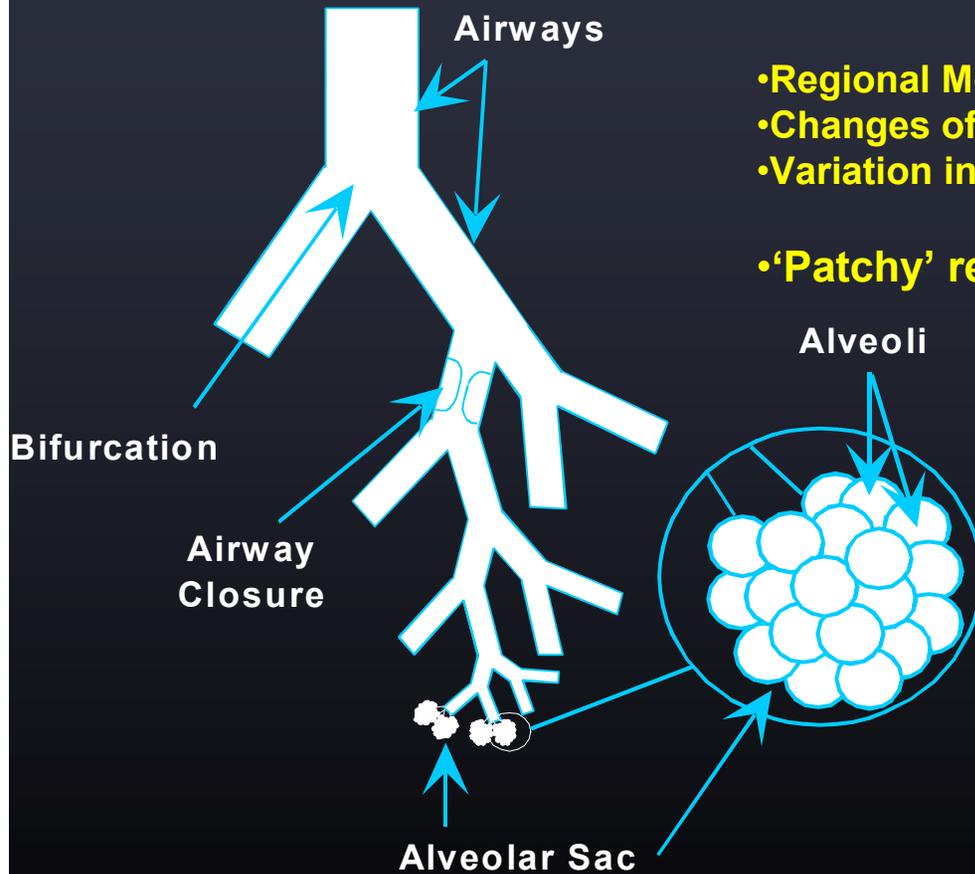
- (A) At FRC the lower region is less expanded, but more compliant so it receives larger portion of ventilation
- (B) At RV the lower lung regions experience airway closure

Airway Closure in Microgravity

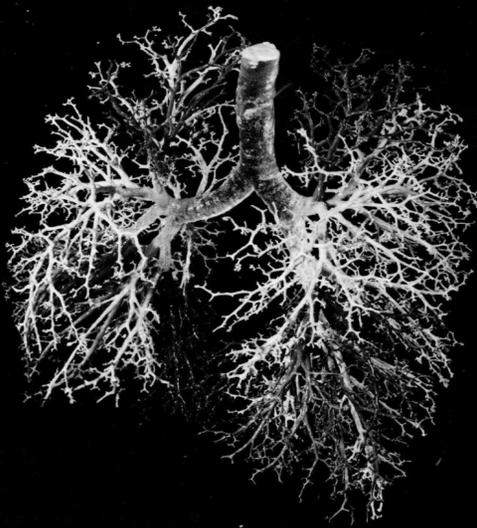
MICROGRAVITY CAUSES:

- Regional Modification of Ventilation
- Changes of Blood Perfusion
- Variation in Lung Capacity

- ‘Patchy’ regions of airway collapse

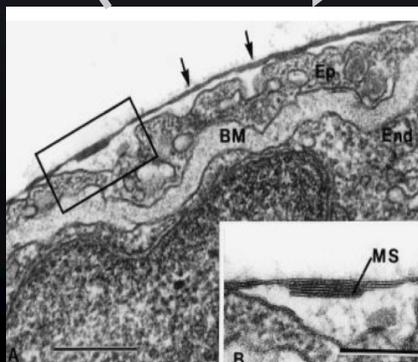
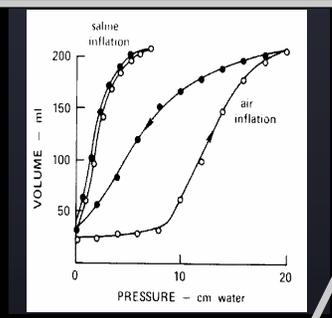
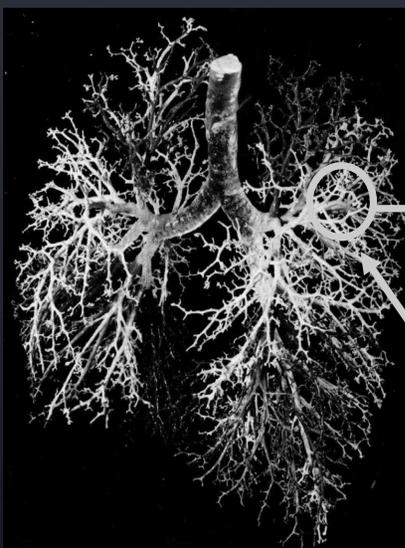


Related Terrestrial Syndromes



- **Infant Respiratory Distress Syndrome**
- **Acute Respiratory Distress Syndrome**
- **Ventilator-Induced Lung Injury**

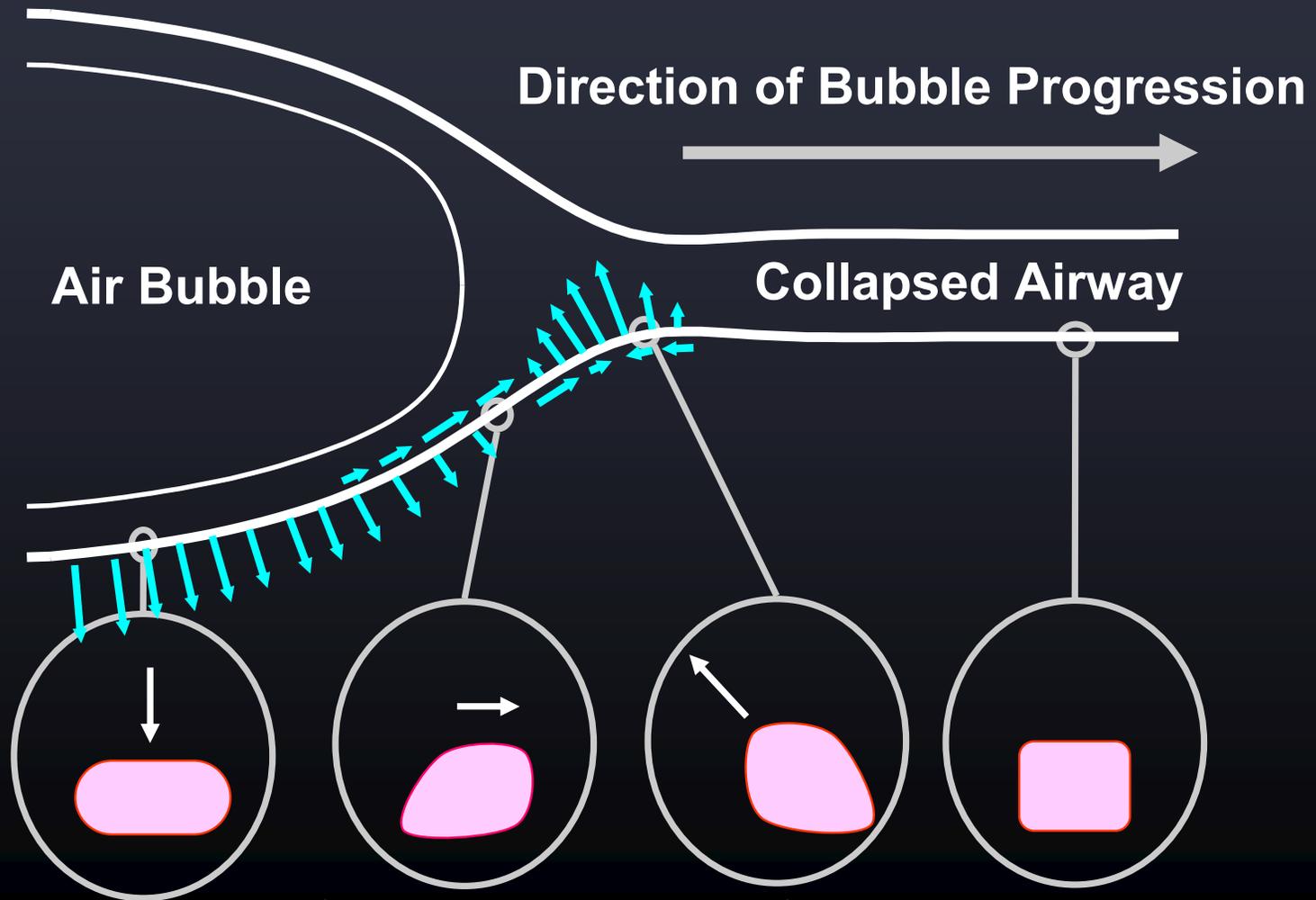
Pulmonary Multiscale Interactions



Motivation

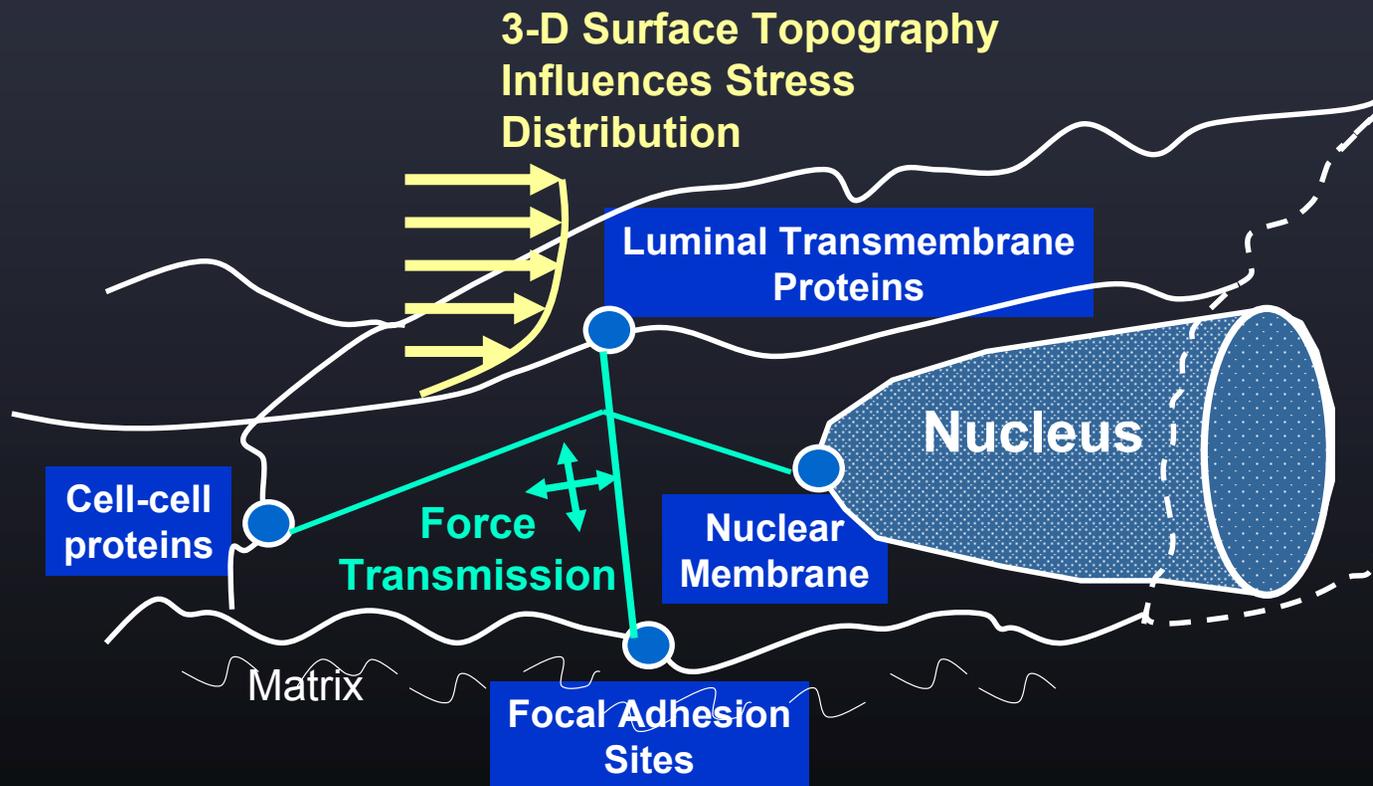
Our goal is to determine the cause of reopening-induced damage, and the surfactant properties and airway reopening strategies that will allow pulmonary airways to be opened with minimal damage to the lung.

Stresses in Airway Reopening



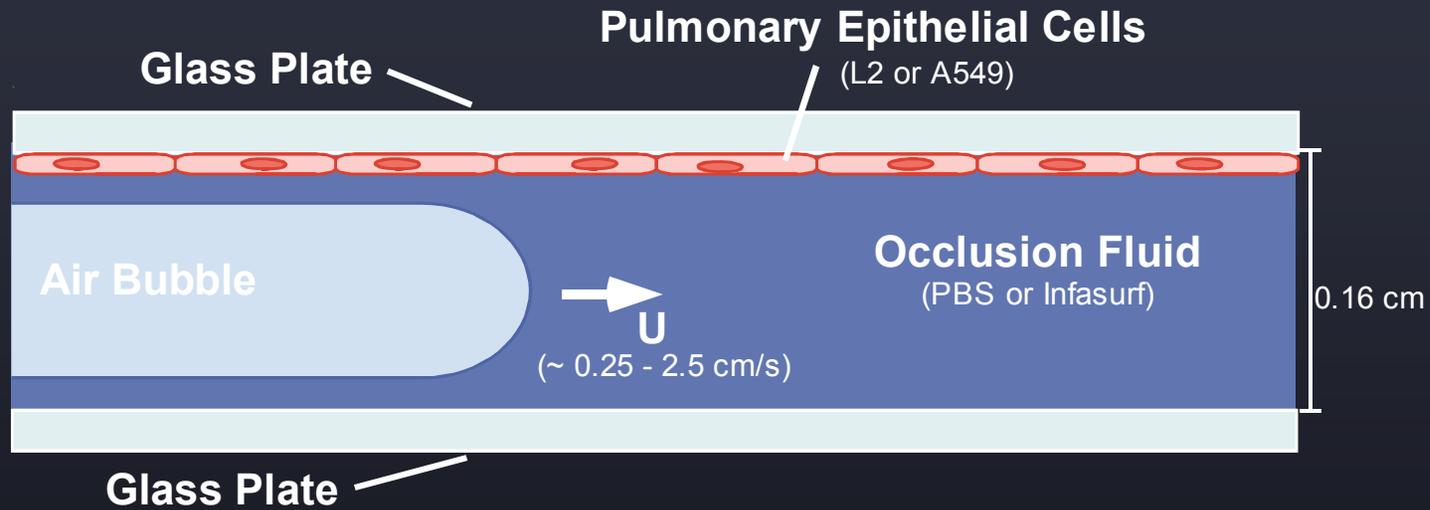
Gaver, Jensen, Halpern and Grotberg, *J. Fluid Mech.*, 1996

Mechanisms of Cell Mechanotransduction and Damage



Adapted from Davies,
Physiol. Rev., 1995

Cell Culture Experiments



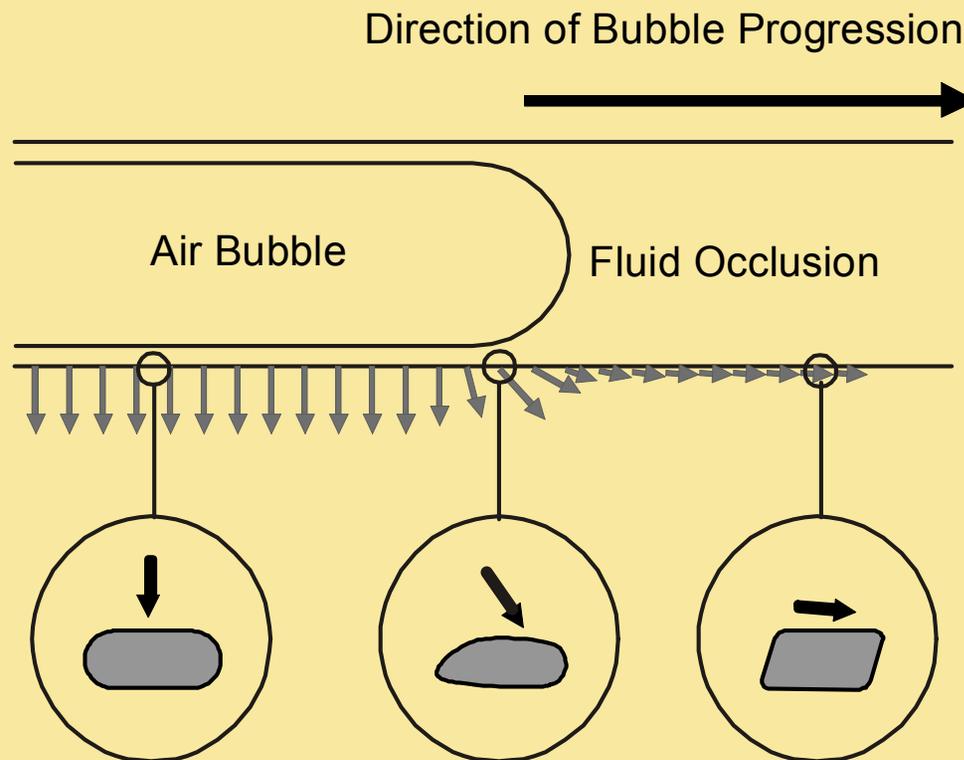
Lung epithelial cells were:

- Cultured in an idealized model of small airways,
- Exposed to a moving finger of air under reopening conditions,
- Examined for cellular trauma.

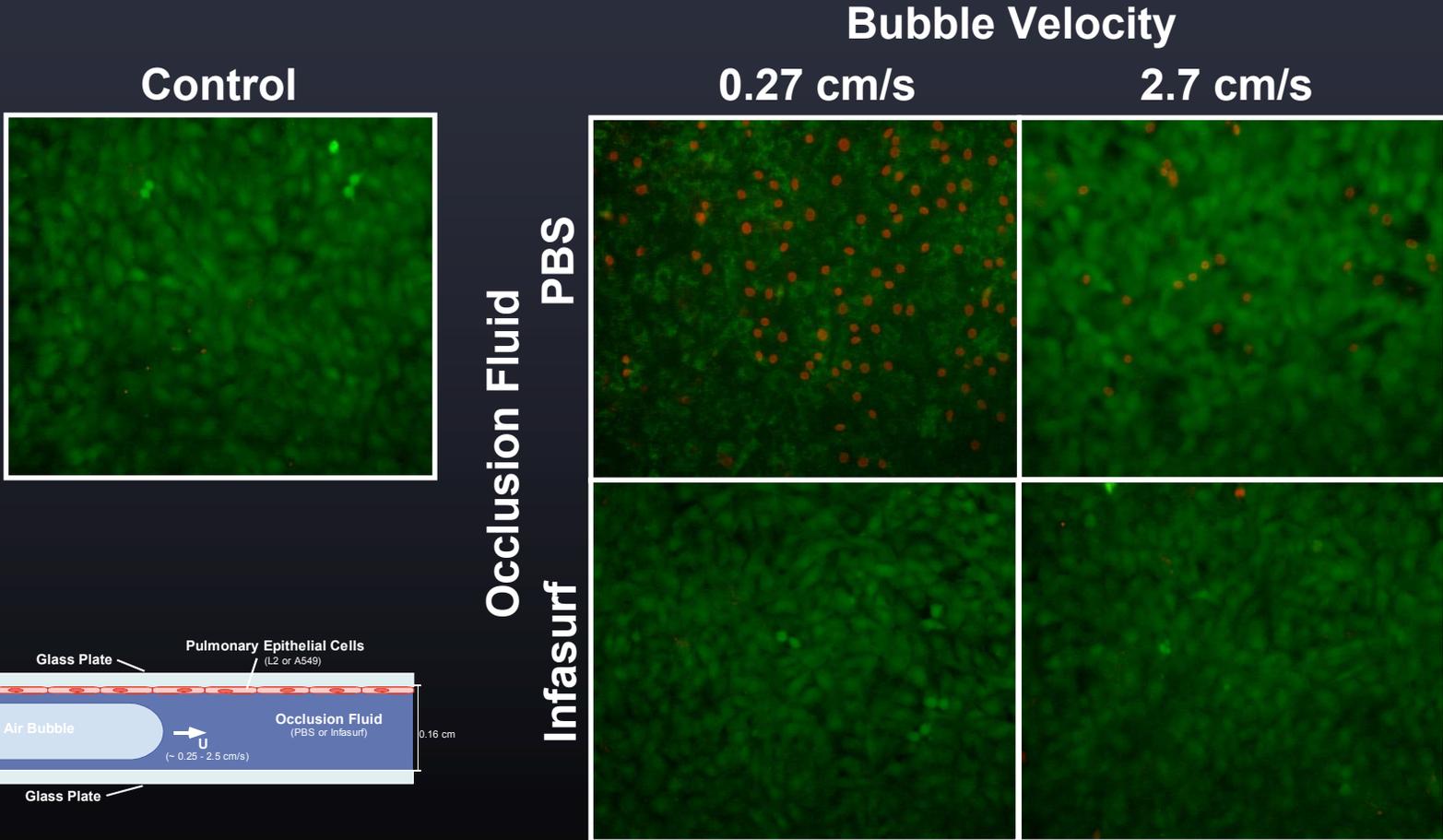
Methods – Variable Velocity

- Lung epithelial cells (CRL-149, ATCC) cultured to confluence on glass microscope slides.
- The channel dimensions were 2.5 x 7.0 x 0.17 cm.
- **Two velocities** (0.27 and 2.7 cm/s) were assessed.
- Two occlusion fluids were assessed:
 - phosphate buffered saline (PBS) and
 - 1 mg/mL Infasurf (ONY, Inc., Buffalo, NY) in PBS.
- Cellular trauma was quantified using fluorescent staining (Live/Dead Kit, Molecular Probes).

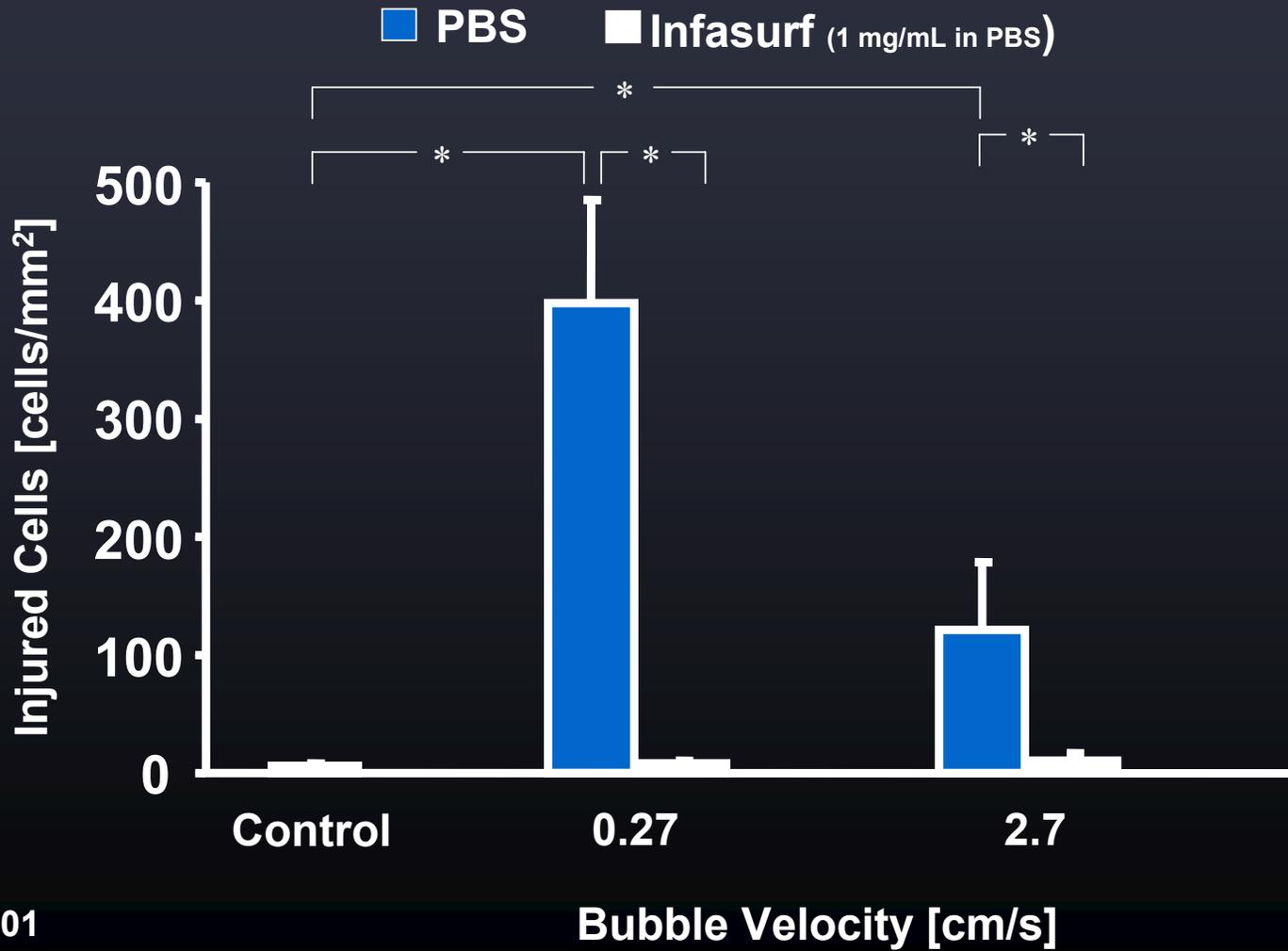
Stress Field – Rigid Channel



Injury by a Single Bubble Progression (L2 cells, Live/Dead Kit)

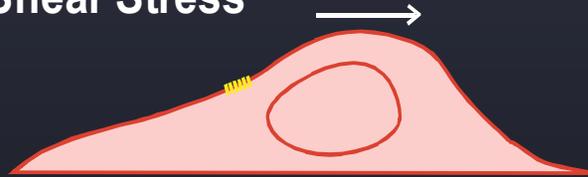


Injury by a Single Bubble Progression (L2 cells, Live/Dead Kit)

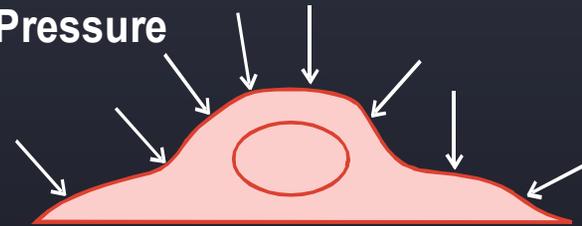


Mechanisms of Cell Membrane Wounding

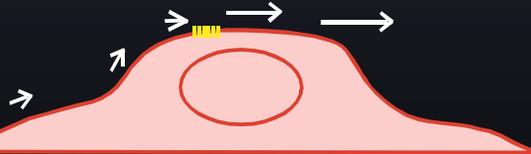
Shear Stress



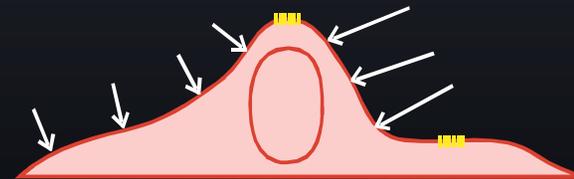
Pressure



Shear Stress Gradient

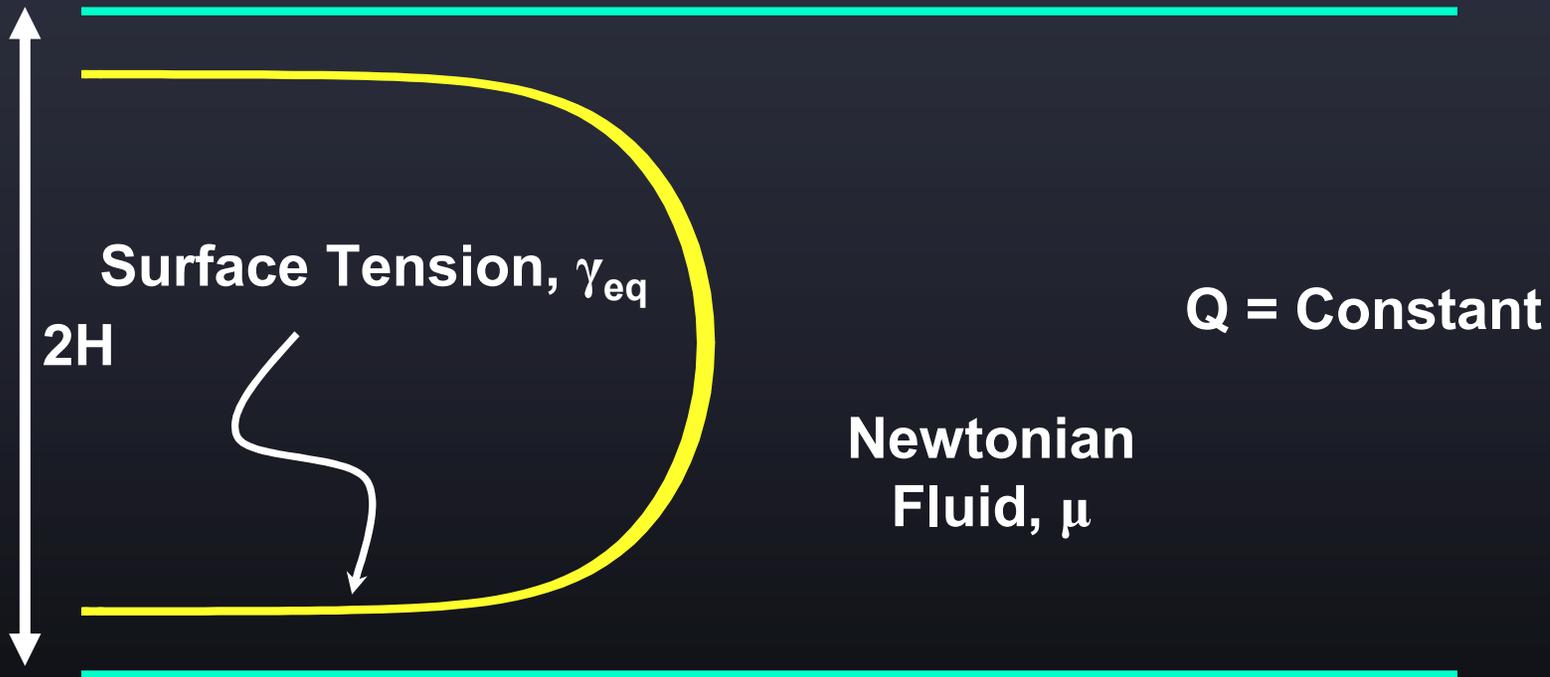


Pressure Gradient



The Flow Model

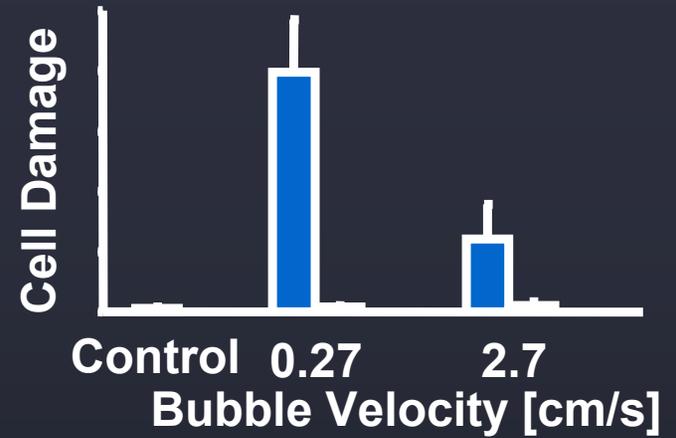
Steady Flow of a Semi-Infinite Bubble in a Channel



Governing Parameter:

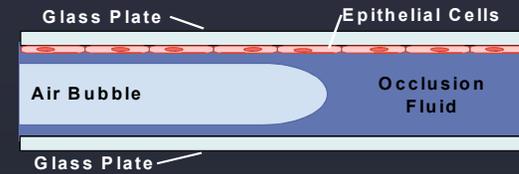
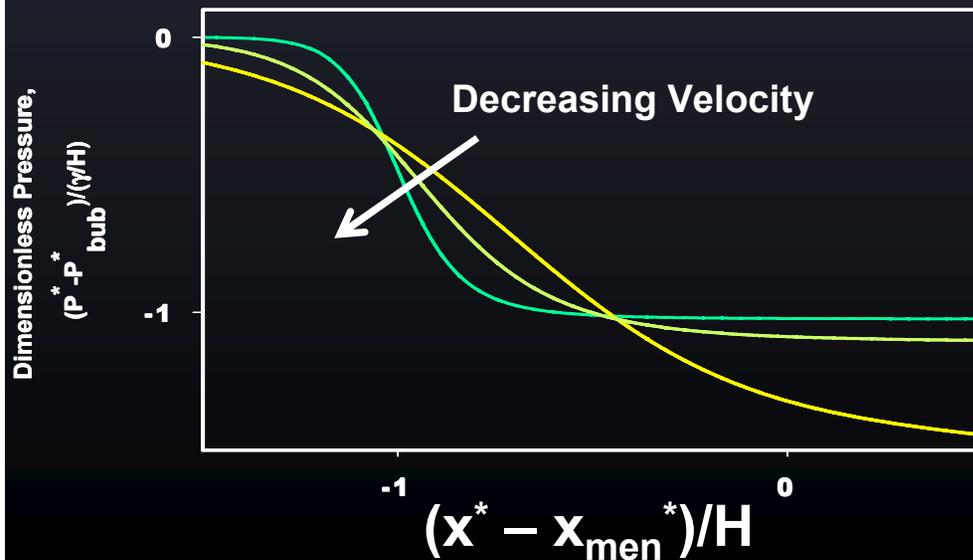
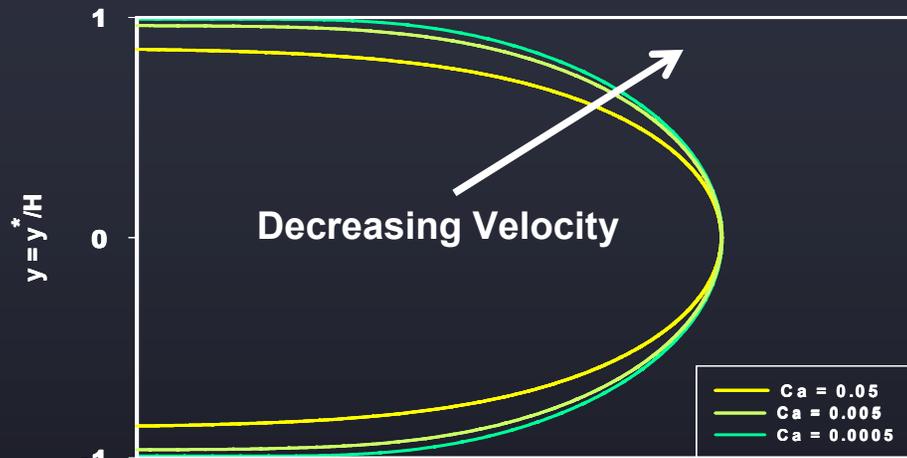
$$Ca = \frac{\mu U}{\gamma_{eq}}$$

Predictions



Fluid	Speed	τ_s (dyn/cm ²)	$\Delta\tau_s$ (dyn/cm ²)	ΔP (dyn/cm ²)	f (μm)
Saline	Slow	15.5	9.2	340	1.4
Saline	Fast	34.3	10.1	170	6.0
Infasurf	Slow	7.9	3.4	89	2.7
Infasurf	Fast	17.5	3.8	44	11.6

Predictions of Cell Normal Stresses



1. Film Thickness decreases with decreasing velocity
2. The pressure gradient on the cell surface increases with decreasing velocity

Investigations of the Applied Stress Duration

The variable velocity experiments induce stresses on cells that are not of constant duration.

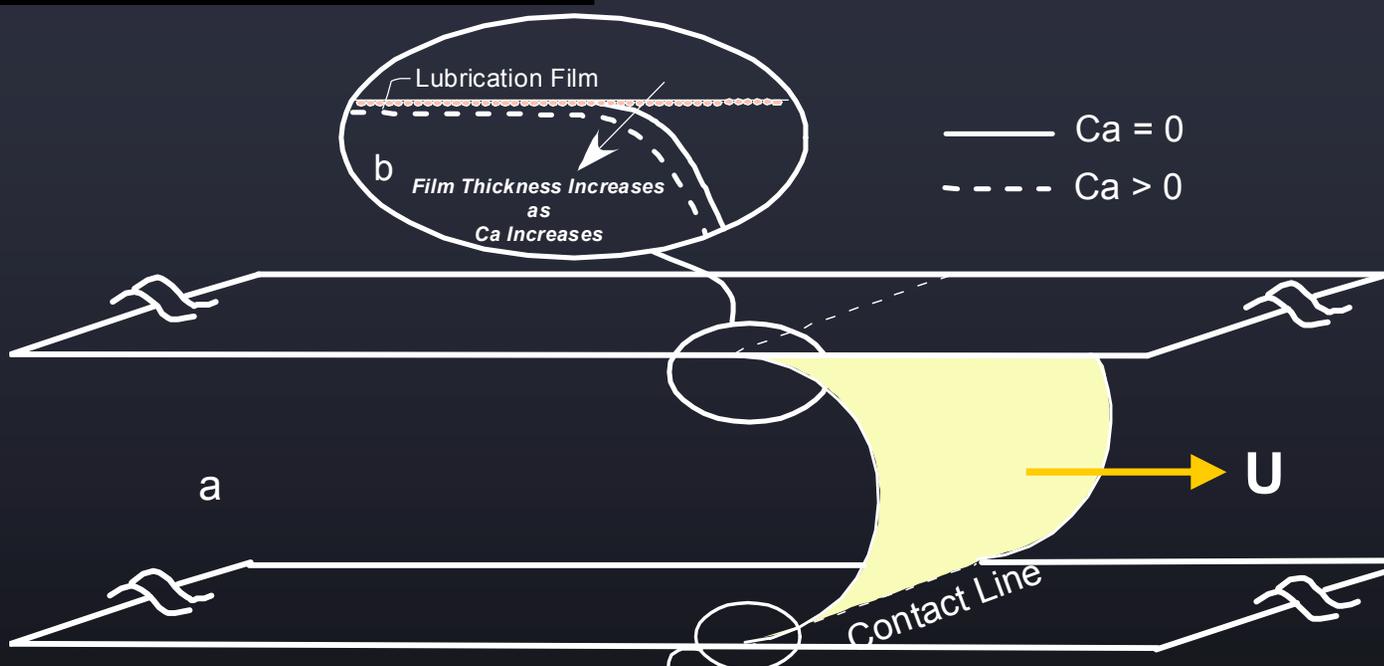
Hypothesis:

The slow velocity experiments may induce greater damage because of the increased exposure time.

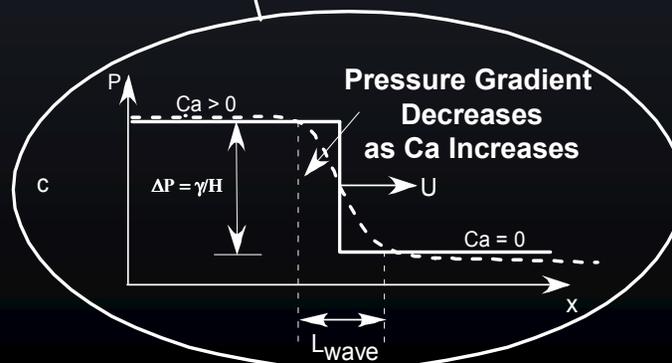
Methods – Constant Velocity

- Human Pulmonary Epithelial Cells (A549, ATCC) cultured to confluence on glass microscope slides.
- The channel dimensions were 2.5 x 7.0 x 0.17 cm.
- A **single velocity** (0.34 cm/s) was applied.
- **Two viscosities** were used
 - $\mu = 8 \times 10^{-3} \text{ g}/(\text{cm s})$ (PBS)
 - $\mu = 8 \times 10^{-2} \text{ g}/(\text{cm s})$ (PBS + 14% Dextran)
- Cellular trauma was quantified using fluorescent staining (Live/Dead Kit, Molecular Probes).

Traveling-Wave Behavior



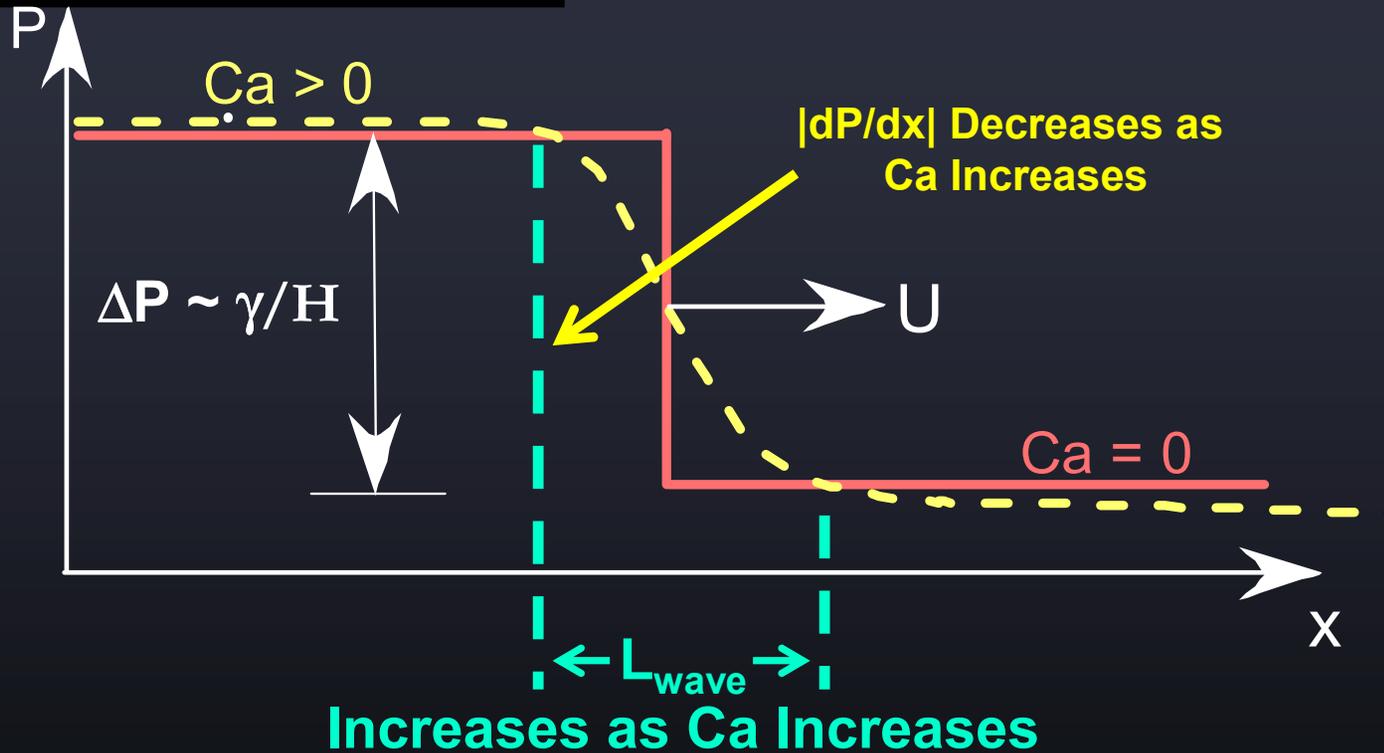
$$Ca = \frac{\mu U}{\gamma}$$



Pressure Field Near Contact Line

Traveling-Wave Behavior

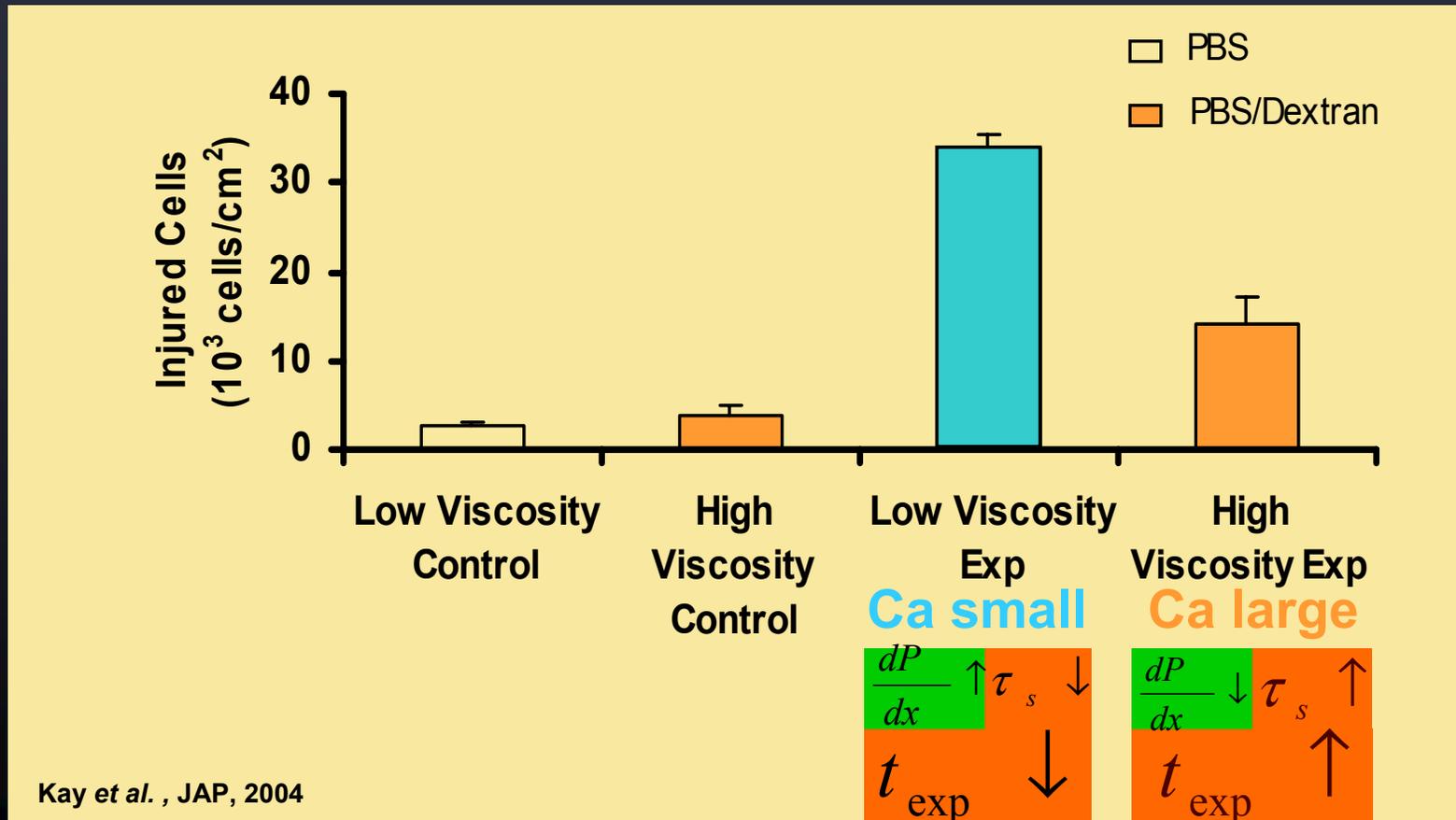
$$Ca = \frac{\mu U}{\gamma}$$



$$\Delta t_{exp} = \frac{L_{wave}}{U} \propto \frac{H\mu^{0.29}}{U^{0.71}\gamma^{0.29}}$$

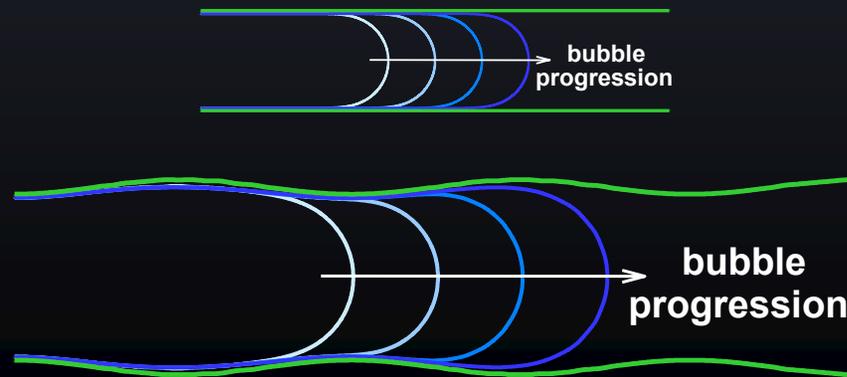
Pressure Gradient, not Exposure Duration, Determines Damage

$$U = 0.34 \text{ cm/s}, \mu_{\text{Dextran}} = 10\mu_{\text{PBS}}$$

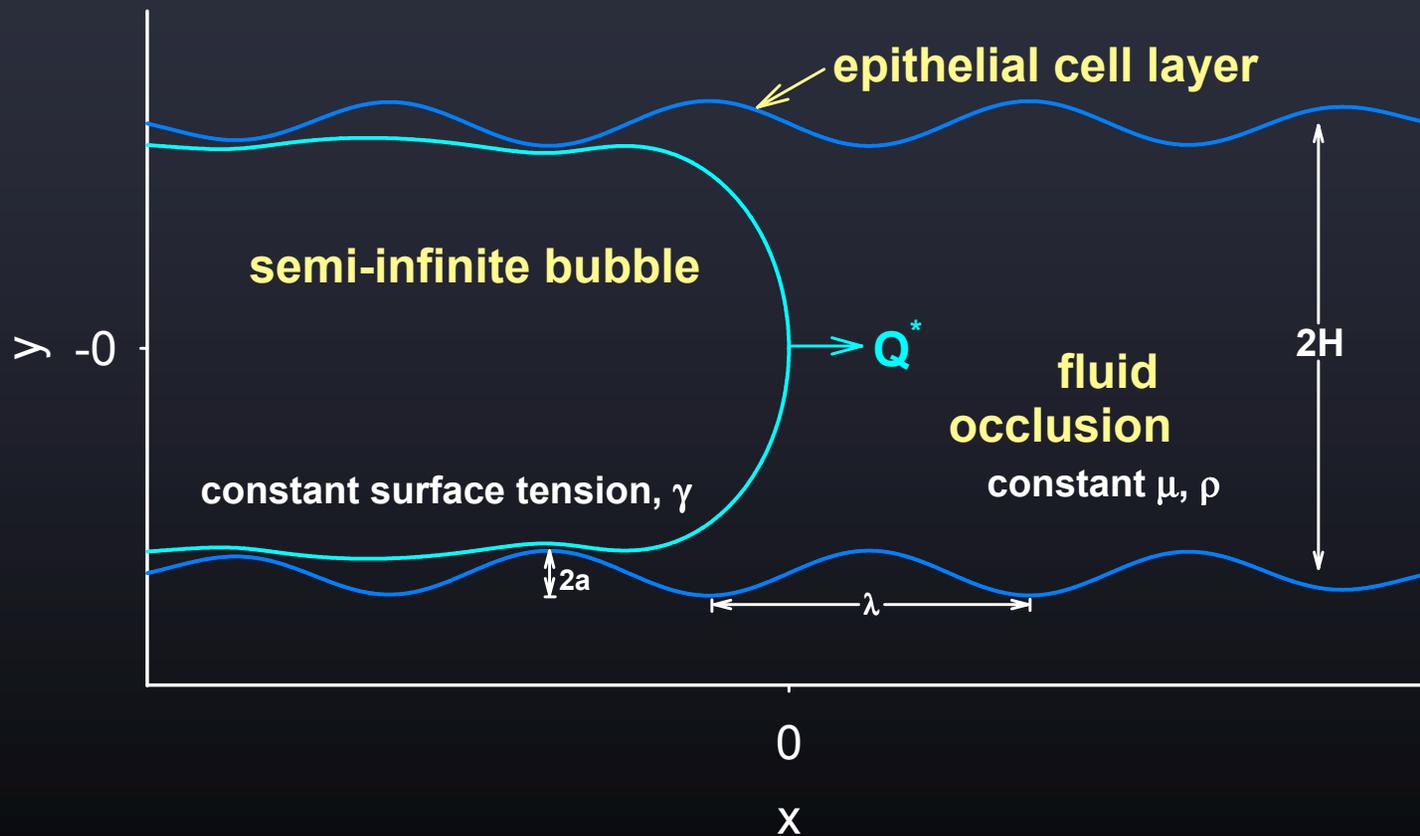


Investigations of Topography

- Our system is modeled to isolate the influence of epithelial topography on the following components of the stress cycle during airway reopening:
 - shear stress and shear stress gradient
 - normal stress and normal stress gradient



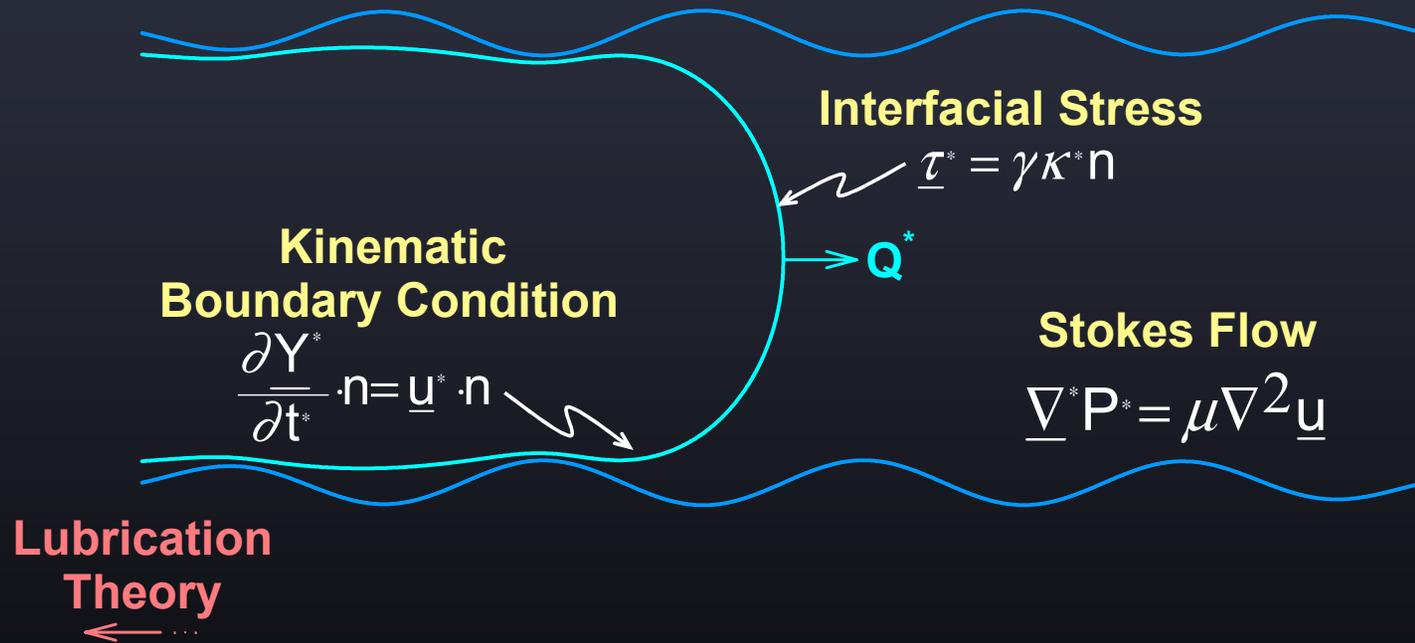
Computational Model



- Geometric Parameters: $\varepsilon = a/H$ $\Lambda = \lambda/H$

Computational Model

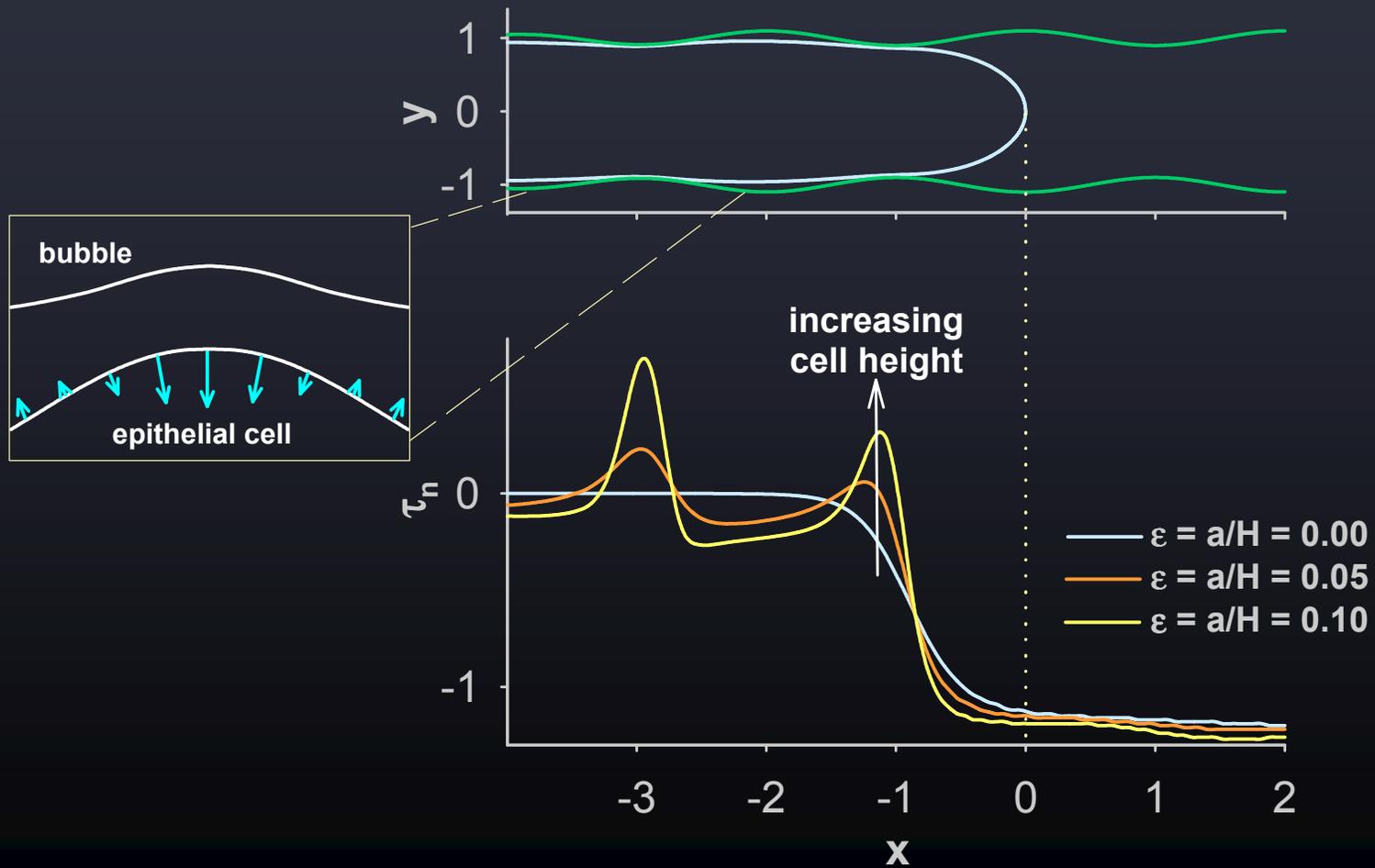
Boundary Element Method



- **Governing Parameter:** $Ca_Q = \frac{Q^* / 2H}{\gamma l \mu}$

Normal Stress Distribution

$$\lambda/H = 2, Ca = 0.01$$



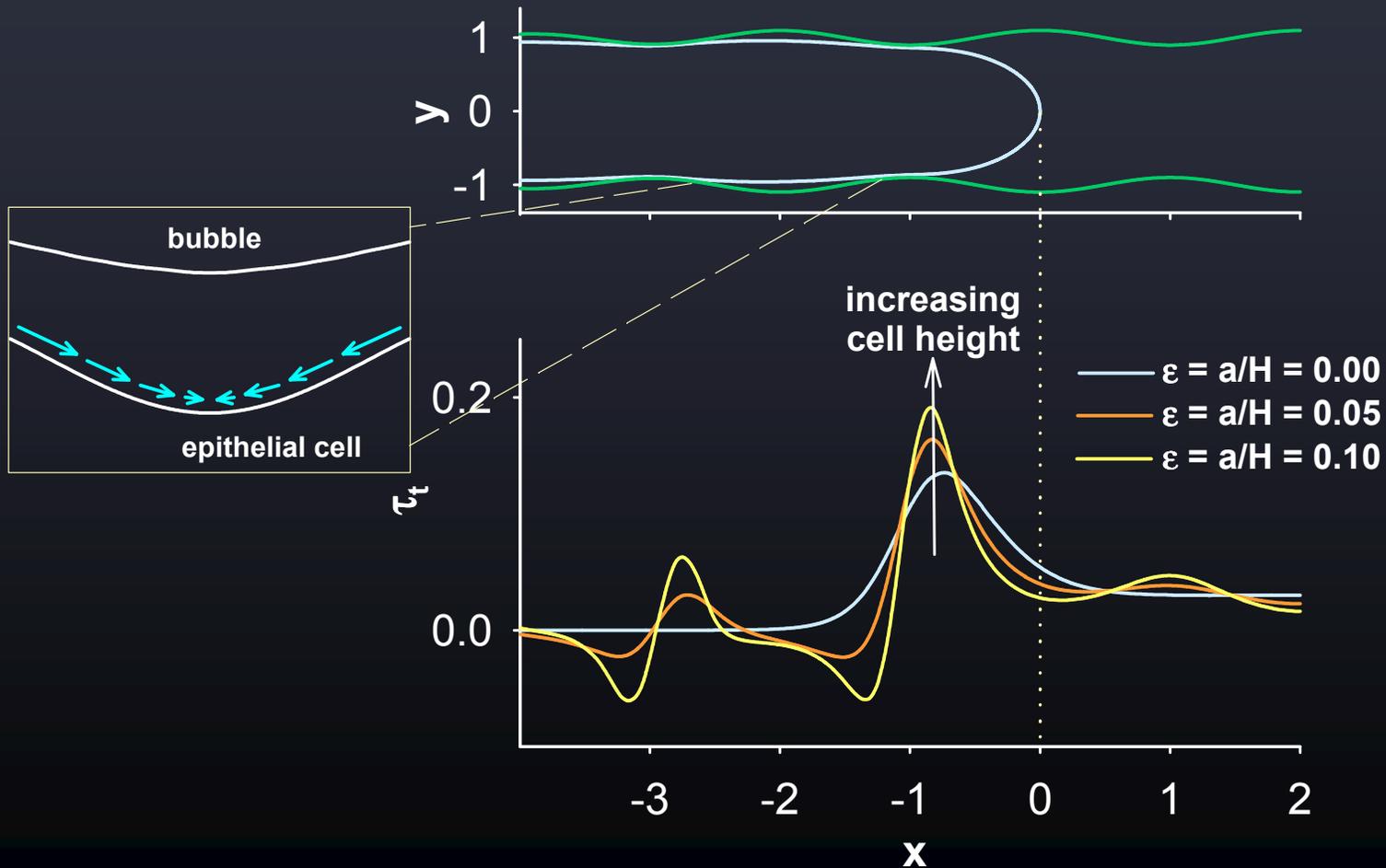
Normal Stress Distribution

$a/H = 0.1, \lambda/H = 2, Ca = 0.01$



Tangential Stress Distribution

$\lambda/H = 2, Ca = 0.01$



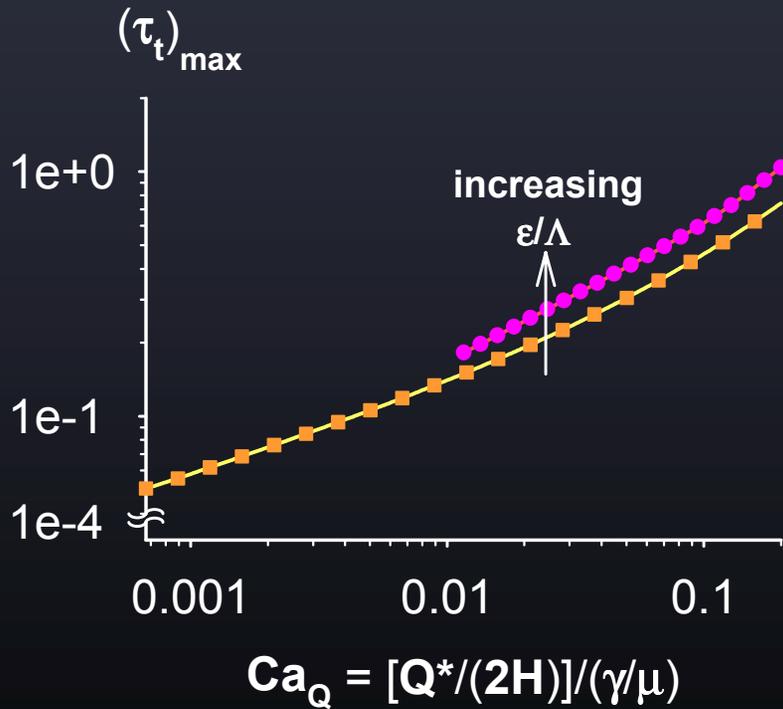
Tangential Stress Distribution

$a/H = 0.1, \lambda/H = 2, Ca = 0.01$

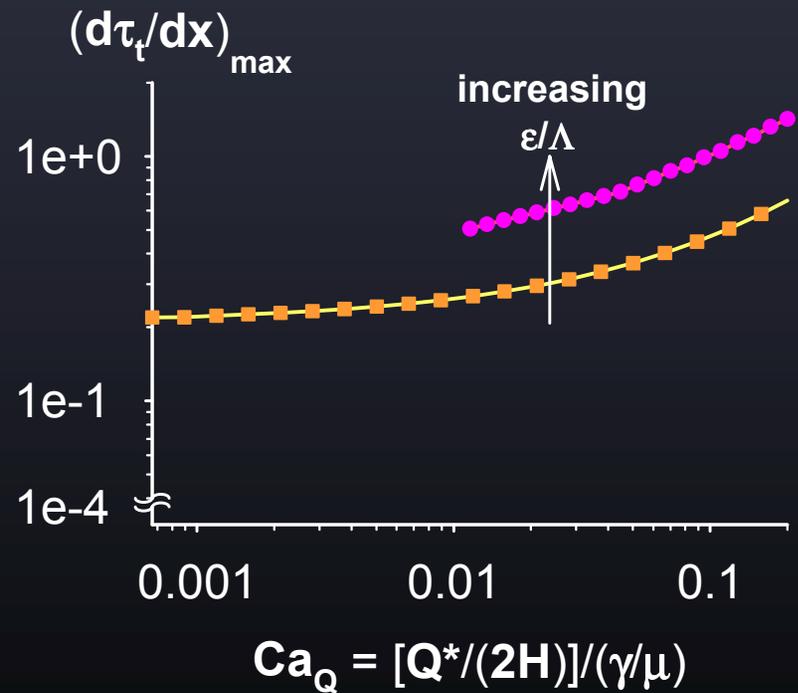


Ca_Q vs. Tangential Stress and Stress Gradient $\epsilon/\Lambda = 0.05$

Tangential Stress



Tangential Stress Gradient

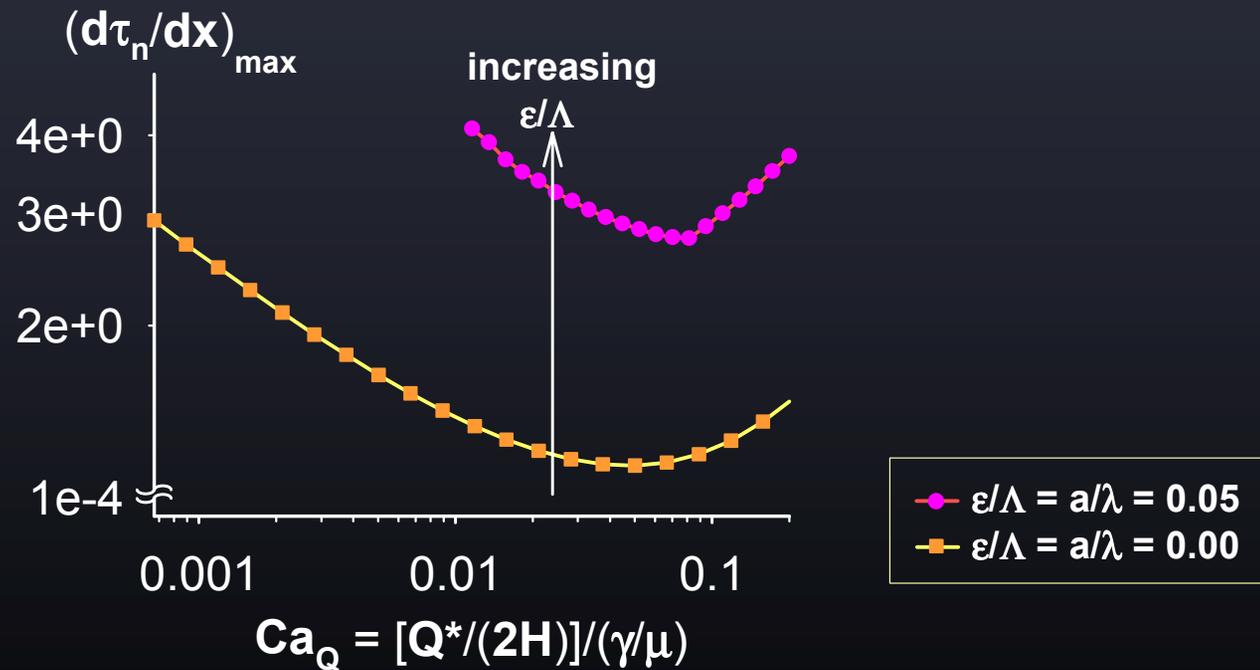


● $\epsilon/\Lambda = a/\lambda = 0.05$
■ $\epsilon/\Lambda = a/\lambda = 0.00$

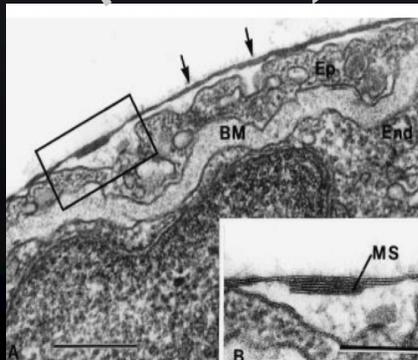
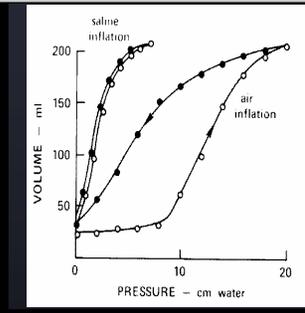
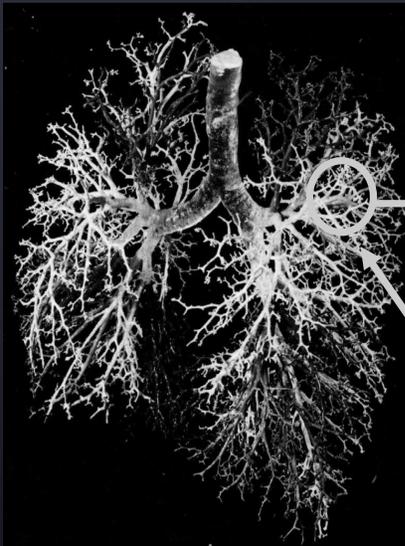
Ca_Q vs. Normal Stress Gradient

 $\epsilon/\Lambda = 0.05$

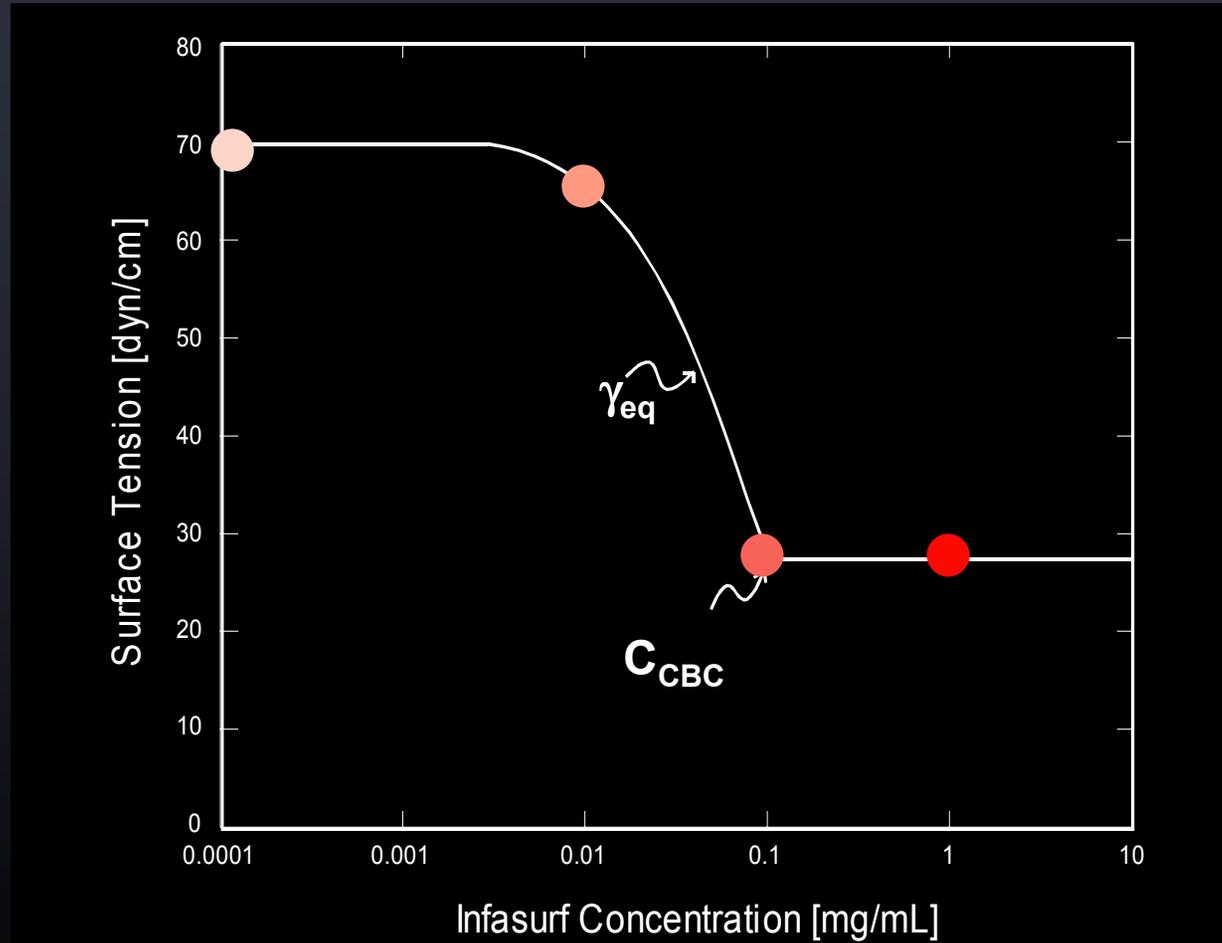
Normal Stress Gradient



Surfactant Effects



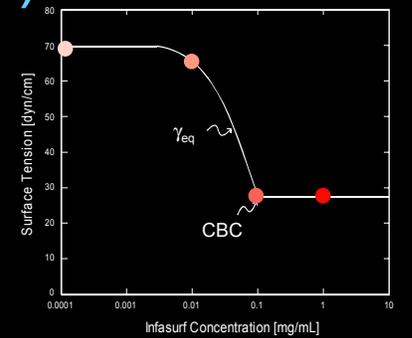
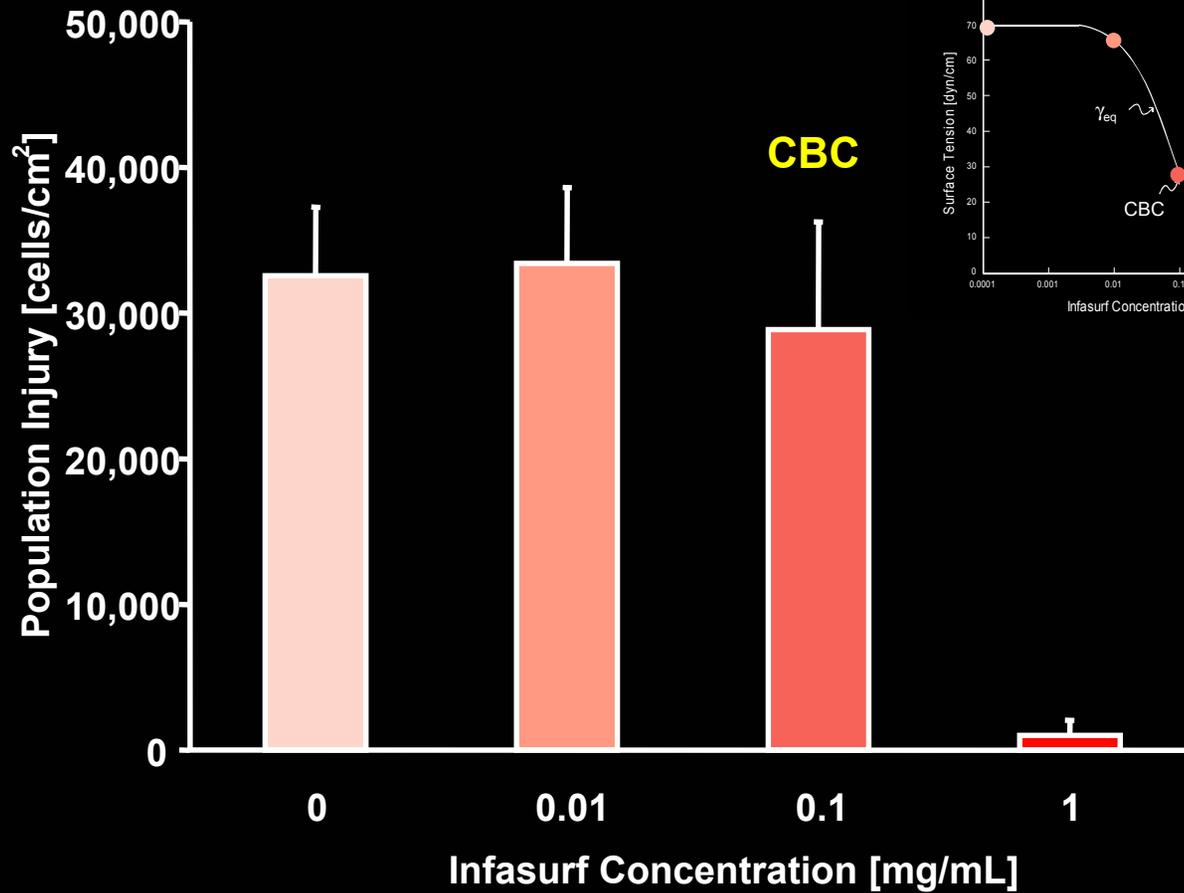
Equilibrium Equation of State (Infasurf)



Ghadiali, S. N. and D. P. Gaver, III (2000). *J. Appl. Physiol.* 88: 493-506.

Influence of Surfactant Concentration

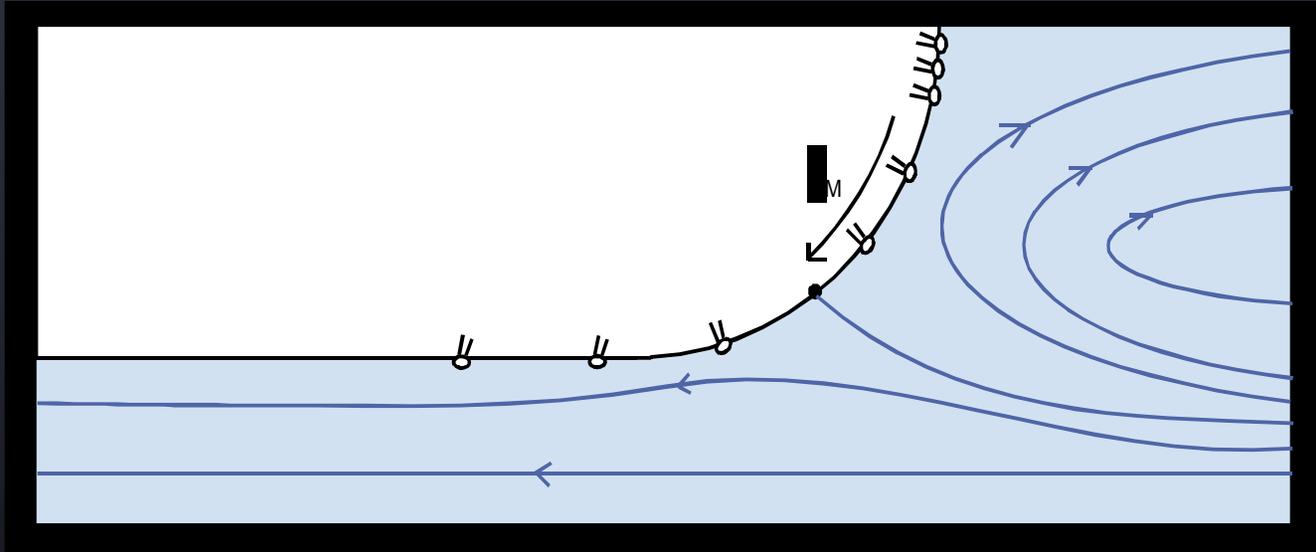
(A549 cells, Live/Dead Kit, 0.25 cm/s)



Correlation of Stress and Injury

Infasurf (mg/mL)	Speed (cm/s)	Injury (cells/cm ²)	τ_s (dyn/cm ²)	$\Delta\tau_s$ (dyn/cm ²)	ΔP (dyn/cm ²)	
0	0.25	++	13.1	4.8	163	✓
0.01	0.25	++	12.8	4.6	154	✓
0.1	0.25	++	7.1	1.9	48	?
1	0.25	-	6.7	1.8	44	✓

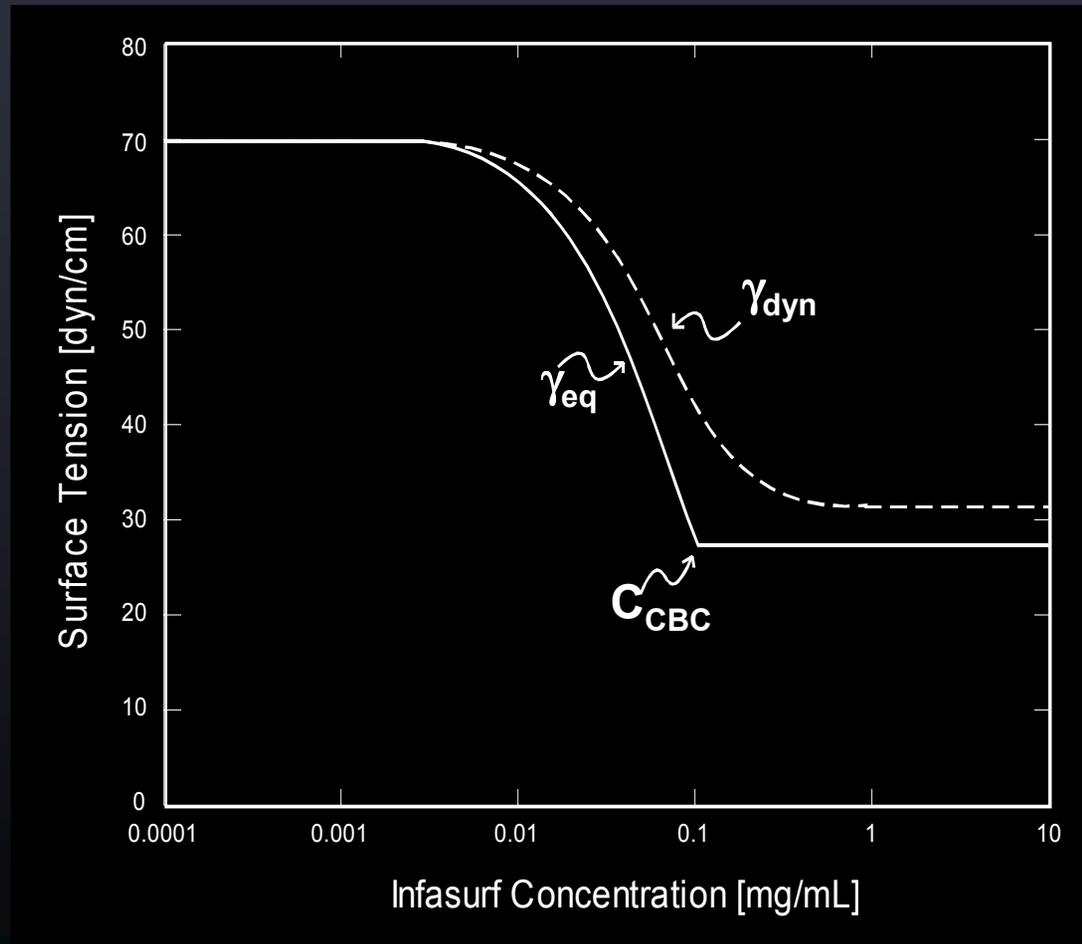
Influence of Non-Equilibrium Behavior of Infasurf



Non-equilibrium behavior:

- ❖ produces dynamic surface tensions that are greater than the equilibrium surface tension,
- ❖ creates non-equilibrium surface tension that causes film-thinning.

Dynamic Surface Tension of Infasurf



Ghadiali, S. N. and D. P. Gaver, III (2000). *J. Appl. Physiol.* 88: 493-506.

Conclusions

- Combined experiments and computational investigations allows us to estimate the mechanical stresses that damage epithelial cells during reopening
- The damaging effects from reopening are likely to be due to a large **pressure gradient** from the traveling air-liquid interface
- **Topological effects** can increase the magnitude of deleterious stresses.

Non-equilibrium surface-tension effects may increase damage unless concentrations are large.

NASA: NAG3-2734
NIH: P20 EB001432
NSF: BES-9978605